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Structural and functional study of the rat distal nephron: Effects of potassium adaptation and depletion

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Structural and functional study of the rat distal nephron: Effects of potassium adaptation and depletion. To examine the relationship between tubular transport of potassium and cell structure in segments of the superficial distal nephron, we performed potassium transport and quantitative electron microscopic studies in rats after potassium adaptation and potassium depletion. In distal nephrons continuously microperfused in vivo, potassium adaptation stimulated potassium secretion by 200%. Microperfused distal convoluted tubules (earliest portion of accessible distal nephron) did not, however, secrete potassium in potassium-adapted animals. Morphometric analysis of the distal convoluted tubule also revealed no detectable effect of potassium diet on the structure of the distal cell type. In contrast, examination of the connecting tubule and the initial collecting tubule of the distal nephron demonstrated a striking increase in basolateral membrane in potassium-adapted animals. This change was limited to the connecting tubule cell and the principal cell type. No structural change of the intercalated cell type in either segment was associated with altered potassium transport. We conclude that cells of the distal convoluted tubule do not secrete potassium. Functional and morphologic evidence suggests that potassium is secreted by the connecting tubule cell and the principal cell of the connecting tubule and the initial collecting tubule, respectively.

Etude structurale et fonctionnelle du néphron distal du rat: Effets de l'adaptation au potassium et de la déplétion en potassium. Afin d'étudier la relation entre le transport tubulaire de potassium et la structure cellulaire dans des segments du néphron distal superficiel, le transport de potassium a été étudié et la microscopie électronique quantitative réalisée chez des rats après adaptation au potassium et déplétion en potassium. Dans les néphrons distaux microperfusés en continu in vivo, l'adaptation à potassium stimule la sécrétion de potassium par un facteur deux. Chez les animaux adaptés à potassium, cependant, les tubes contournés distaux microperfusés ne sécrètent pas de potassium. L'analyse morphométrique du tube contourné distal ne montre pas d'effet décelable du contenu en potassium de l'alimentation sur la structure des cellules tubulaires distales. Par contre l'étude du tube connecteur et de la partie initiale du tube collecteur montre une augmentation considérable des membranes baso-latérales chez les animaux adaptés à potassium. Cette modification est limitée aux cellules de type connecteur et de type principal. Aucune modification structurale des cellules intercalaires n'a été observée dans ces deux segments au cours de modifications du transport de potassium. Nous concluons que les cellules du tube contourné distal ne sécrètent pas de potassium. Il existe des arguments fonctionnels et morphologiques en faveur d'une sécrétion de potassium par les cellules du tube connecteur et les cellules principales du tube collecteur initial.

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In recent years there has been a growing appreciation that the proximal and distal subdivisions of the nephron can be further subdivided with respect to structural features [1–5], transport characteristics [6–10], and hormone response [2, 9, 11]. This work suggests that the structurally distinctive cell types found along the nephron may have very different physiologic roles. In the case of epithelia possessing more than one cell type, enzyme localization [12] and structural studies [13–16] indicate that various cell types may have very different functions.

The *superficial* distal nephron, between the macula densa and the confluence of two tubules[1], can be divided into four successive segments on the basis of cell morphology [2–5]. The subcapsular macula densa region (DCT_a) contains one cell type. These cells are not found in contact with the renal capsule and, therefore, are not accessible to micropuncture. Figure 1 is a schematic representation of cell types encountered in the distal nephron in micropuncture studies. The first accessible segment, the *distal convoluted tubule* (DCT_b, "early distal tubule"), is a homogeneous epithelium composed of the distal cell type. Distal cells have extensive basolateral membrane interdigitations (Fig. 1). Interdigitating cell processes are filled with elongated

[1] Traditionally, this segment of the nephron has been called either the distal convoluted tubule or the distal tubule in micropuncture studies. Segments of the superficial distal nephron (DCT) from the macula densa to the confluence with another tubule have been identified by Morel, Chabardes, and Imbert [2]. The subscripts a, b, g, and l represent ascending limb, bright, granular, and light segments, respectively.

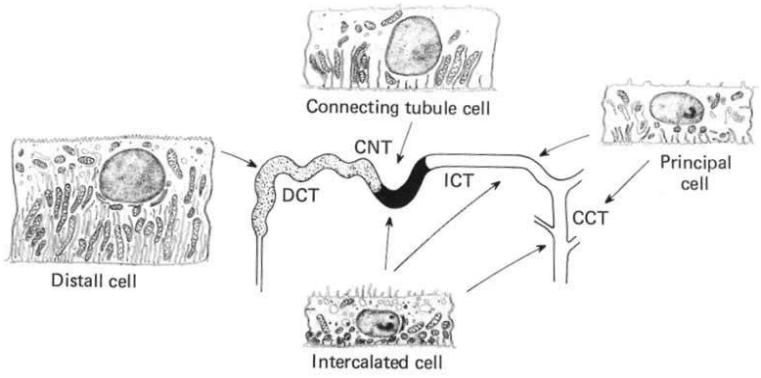

Fig. 1. *Schematic representation of the rat superficial distal nephron.* The *arrows* indicate the location of each cell type within the nephron. The three segments studied are distal convoluted tubule (DCT), the connecting tubule (CNT), and the initial collecting tubule (ICT). This figure was drawn from our electron microscopic observations. CCT is cortical collecting tubule.

mitochondria. Numerous short microvilli characterize the luminal membrane. The third segment, called the *connecting tubule* (DCT_g, transitional, intermediate, or "mid distal tubule") is extremely short (150- to 200-μm long) in superficial nephrons of rat [4] and contains connecting tubule cells (Fig. 1) and intercalated cells. Connecting tubule cells possess true basolateral membrane infoldings. Elongated mitochondria are often located within these infoldings, but some infoldings are devoid of mitochondria. The apical membrane is relatively smooth and has few distinct microvilli [3, 5, 21]. In the final segment of the distal nephron, the *initial collecting tubule* (DCT_1, referred to as "late distal tubule" in the micropuncture literature), two cell types can be identified: principal cells (light cells) and intercalated cells (dark cells). Principal cells have a cytoplasm with few organelles, ovoid mitochondria, which are not regularly associated with the basolateral membrane infoldings, a smooth apical membrane, an absence of cytoplasmic vesicles, and a centrally located nucleus (Fig. 1). Intercalated cells have an abundance of cytoplasmic organelles, luminal membrane microplicae, numerous apical cytoplasmic vesicles, and a basally located nucleus (Fig. 1).

Investigators have recently observed in cortical and medullary collecting tubules [13–15] a correlation between altered cell morphology and renal tubular function. These studies suggest that principal cells may be involved in potassium secretion and that intercalated cells may mediate potassium reabsorption.

To evaluate the possible contribution of different cell types in the superficial distal nephron to renal potassium transport, we have examined cells of the distal convoluted tubule, the connecting tubule, and the initial collecting tubule segments when potassium secretion was stimulated by potassium adaptation and inhibited by potassium depletion.

Our studies demonstrate that: *(1)* The cells of the distal convoluted tubule segment do not secrete potassium. *(2)* Intercalated cell structure and incidence is not altered when distal nephron potassium transport is modulated by dietary potassium, and *(3)* marked changes in the structure of the connecting tubule cell of the connecting tubule and the principal cell of the initial collecting tubule are associated with increased potassium secretion resulting from potassium adaptation.

Methods

Male Sprague-Dawley rats, each weighing 264 to 356 g, were used in all experiments. Three groups of animals were studied: *(1)* potassium-adapted rats received a Teklad diet containing 15 g of potassium chloride per 100 g of diet (no. 170550) ad lib for 4 to 6 weeks and 0.1 M potassium chloride to drink, *(2)* potassium-depleted rats received a Teklad potassium-deficient diet containing 0.002 g of potassium chloride per 100 g of diet (no. 170555) ad lib for 4 to 6 weeks and distilled water to drink, *(3)* control rats received Purina lab chow (0.96 g of potassium chloride per 100 g of diet) and tap water. All animals were starved for 15 hours before each experiment but were allowed free access to their drinking solution. Animals were anesthetized with Inactin (100 mg/kg body wt, i.p.).

Functional studies. Rats were prepared for micropuncture as described previously by this laboratory [17]. All animals received an i.v. infusion of mammalian Ringer's solution at 1.5 ml/hr · 100 g of body wt. Thirty-two distal nephrons were microperfused in twenty-two animals. Superficial distal

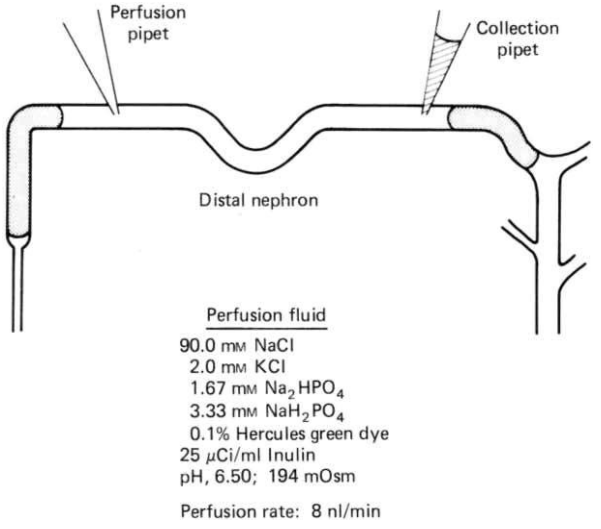

Fig. 2. *Schematic representation of microperfusion experiments.* Composition of perfusion fluid approximates in vivo distal convoluted tubule fluid.

nephrons were perfused in vivo at 8 nl/min with a Hampel microperfusion pump (Frankfurt, a.M. West Germany). The perfusion pipette was placed in the distal convoluted tubule, distal to a castor oil block, and the collection pipette was located in the initial collecting tubule, proximal to a second castor oil block (Fig. 2). The collection rate was varied to maintain both oil blocks in position. The perfusion fluid was made up to simulate distal convoluted tubule fluid (Fig. 2). Tritiated inulin was added to the perfusion solution, and only those tubules where inulin recovery was between 85 and 115% of delivery were accepted. Recovery in 32 tubules averaged 96.9 ± 1.6% and was similar in all three groups of animals. At the end of each experiment, silicone rubber was injected into each microperfused tubule (Canton BioMedical Products, Boulder, Colorado). The kidney was digested in 25% sodium hydroxide, and the silicone rubber casts were teased from the surrounding tissue. The length of the distal nephron, from the macula densa to the confluence, was measured and the position of the perfusion and collection site determined. The average perfused length of all distal nephrons was 1.42 mm. In three instances, the distal convoluted tubule was microperfused. These segments were approximately 250-μm long (in vivo measurement, eyepiece reticle).

Absolute reabsorption or secretion of sodium and potassium, by the distal nephron, was calculated from the amount delivered by the perfusion pipette minus the amount collected by the collection pipette. For example, absolute sodium reabsorption (AR_{Na}) was determined by

$$AR_{Na} = \dot{V}_{in}[Na]_{in} - \dot{V}_{out}[Na]_{out}$$

where $\dot{V}$ is the perfusion rate and subscripts "in" and "out" denote perfusate fluid and collected fluid, respectively. The perfusion rate ($\dot{V}_{in}$) was determined each day in vivo. The perfusion rate was calculated in vivo by the equation

$$\dot{V}_{in} = \frac{[inulin]_{out}}{[inulin]_{in}} \cdot \dot{V}_{out}$$

Isotopic counting—$[inulin]_{in}$ (inulin concentration in the perfusate), $[inulin]_{out}$ (inulin concentration in the collected fluid)—was done by a Searle Analytical 92 in a 77% solution of Aquasol (New England Nuclear, Boston, Massachusetts) in water, for 10,000 total counts. The collection rate ($\dot{V}_{out}$) was calculated by measuring the volume of a timed collection in a constant bore glass capillary tube that was calibrated with a solution containing tritiated inulin. Potassium and sodium concentrations in tubule collections were determined by a helium glow photometer (American Instrument Co., Silver Springs, Maryland).

Structural studies. Animals in this group were not studied by micropuncture, but they received exactly the same dietary pretreatment as animals in the functional studies. Eighteen animals, six in each group, were examined by morphometric techniques. After anesthesia, an abdominal incision was made, and the descending aorta and the left renal vein were exposed. An 18-gauge needle was inserted into the aorta, distal to the renal artery. The left renal vein was cut, and the descending aorta was occluded proximal to the renal artery (method I, Manusbach [1]). Initially the left kidney was perfused at a pressure of 150 cm H_2O [1] with mammalian Ringer's solution (37° C) containing 3% T-40 dextran. When the kidney was cleared of blood (less than 5 sec), it was perfused for 5 min with Karnovsky's fixative (37° C) [19], diluted 1:3 with 0.1 M sodium cacodylate buffer (pH, 7.2) and containing 0.1 M sucrose and 3% T-40 dextran. Five blocks of tissue, 1 to 2 mm^3, were randomly selected from the superficial cortex of each kidney and postfixed for 2 to 3 hours in Karnovsky's fixative (full strength) containing 0.1 M sucrose. Following fixation, the tissue was washed in 0.1 M sodium cacodylate buffer (pH, 7.2) with 0.1 M sucrose added. The blocks were postfixed for 1 hour in 1.33% osmium tetroxide buffered with 0.2 M s-collidine (pH, 7.2), and then washed in 0.1 M s-collidine. The tissue was

stained en bloc with uranyl acetate oxylate [20], washed again with 0.1 M s-collidine buffer, dehydrated in graded series of ethanol, and embedded in Epon 812.

To determine whether perfusion pressure influenced the morphometric data, we fixed several kidneys by dripping the fixative described above onto the surface of the kidney in vivo [1]. This method has previously been found to adequately fix the superficial cortical tubules while normal renal blood flow is maintained [1]. The results of the morphometric analysis were similar with both fixation procedures.

Thick sections (0.5 to 1.0 μm) from each block were cut on an LKB Huxley microtome and stained with a methylene blue-azure II stain for light microscopy. It was our intention to examine distal nephrons that were available from micropuncture. Therefore, we selected distal nephrons that were in direct contact with the renal capsule. Two to four distal nephron segments cut in perfect cross-sections from each block of tissue were selected and thin-sectioned. The sections were stained with saturated aqueous uranyl acetate and lead citrate and examined with a Zeiss EM 10 B electron microscope. Distal nephrons were classified as distal convoluted tubule, connecting tubule, or initial collecting tubule based on cellular morphology. General criteria for identification of each segment was presented in the Introduction. Although the distal convoluted tubule is structurally very different from the initial collecting tubule, the connecting tubule shares certain structural features with each of these segments. It is necessary, therefore, to describe our specific criteria for distinguishing the connecting tubule from the adjacent segments.

The connecting tubule contains connecting tubule cells and intercalated cells. The connecting tubule cell is structurally intermediate between the distal cell and the principal cell, having morphologic characteristics of both (Fig. 1). The connecting tubule cell can be distinguished, however, from the distal cell because the connecting tubule cell has a smooth apical membrane and a light-staining apical cytoplasm relatively void of organelles. In addition, the connecting tubule segment has intercalated cells that are not found in the distal convoluted tubule. The connecting tubule cell is also distinctly different from the principal cell. The mitochondria of the principal cell are only rarely associated with the basolateral membranes whereas the connecting tubule cell has many elongated mitochondria associated with infoldings of the basolateral membrane (Figs. 1, 6, 7). Bengele et al [21] and Hayslett et al [22] have previously described the clear distinction between these three segments of the rat superficial distal nephron.

To eliminate any possible bias, we coded all animals, tissue blocks, and micrographs. The code was broken only after all counts and calculations were made. Complete cells cut in cross-section with an intact basement membrane were photographed at × 3400 and enlarged during printing to ×8500. An average of 15 cells of each type per animal were photographed. Stereologic techniques were used to estimate membrane and cell parameters [23]. A grid, composed of semicircular lines with spacing of 1 cm and points 1 cm apart, was projected on each print [24]. The semicircular grid has an isotropic line density that eliminates errors arising from the anisotropic orientation of membranes in epithelia. Surface density of the luminal (S_VLM) and basolateral membrane (S_VBLM) of each cell type was determined by counting the number of intersections of each membrane with the test grid (I_i) and the number of points (P_T) within the cell perimeter. Substitution of I_i and P_T in the following equation estimates the surface density of each membrane, where d is the distance between test points.

$$S_V = \frac{4}{\pi} \cdot \frac{I_i}{dP_T}$$

Boundary length (B) of each membrane was determined by the formula $B = I_i \cdot d$. Cell area (A) was estimated from $A = d^2 \cdot P_T$. The cell point count (P_T) averaged 600 to 1200 for each cell type per animal. Intersections (I_i) averaged 1000 to 5000 for each cell type per animal. This magnitude of counting yielded a counting error of 1.1 to 6.1 percent for each cell type per animal [23]. Cell type incidence (intercalated cells/total number of cells) in the connecting tubule and in the initial collecting tubule was estimated, at a magnification of ×3300, from micrographs in which tubular cross-sections perpendicular to the tubular axis were obtained. A total of 1548 cells were examined to assess intercalated cell incidence.

Microperfused distal convoluted tubules were perfusion-fixed in vivo with half-strength Karnovsky's solution for 5 min. At the end of the experiments, the tubules were filled with microfil, and the puncture sites were carefully noted. After sacrifice, small blocks of tissue surrounding the injected tubule were dissected, fixed, and processed (vide supra). The tubules were serial-sectioned until each puncture site was identified in thick section. Thin

sections were cut 1 μm distal to each puncture site and examined with the electron microscope [18, 22].

Statistical analysis. Preliminary inspection of the data was done by a one-way analysis of variance (ANOVA). If there was a significant difference at $P < 0.05$, the Q (Honestly Significant Difference) test was used to identify the means that were different from control [25]. Levels of significance are expressed based on Q test comparisons. Intercalated cell incidence was compared by the χ^2 (chi-squared) test [25]. All data are expressed as the means ± SEM.

Results

Functional study of the superficial distal nephron. Potassium secretion by the accessible portion of the superficial distal nephron was stimulated by potassium adaptation from 31.8 ± 3.8 pEq/min in control animals to 95.4 ± 15.4 pEq/min (Fig. 3). Sodium reabsorption increased from 212 ± 30 pEq/min in control animals to 458 ± 63 pEq/min in potassium-loaded animals. These results are comparable with previous data demonstrating the ability of the superficial distal nephron in potassium-adapted animals to augment both potassium secretion and sodium reabsorption [17].

Distal nephrons in potassium-depleted animals conserved potassium (Fig. 3). Secretion dropped to 0.5 ± 1.1 pEq/min, a value not different from zero. Sodium transport was not affected by potassium depletion. Tubules of potassium-depleted animals reabsorbed 223 ± 24 pEq/min of sodium compared with 212 ± 30 pEq/min in control.

We conclude that dietary pretreatment was successful in stimulating distal nephron potassium secretion in potassium-adapted animals and in reducing potassium secretion in potassium-depleted animals. These microperfusion studies support previous free-flow micropuncture results that demonstrate the importance of the distal nephron in regulating urinary potassium excretion (reviewed in Refs. 26–28).

Studies of the distal convoluted tubule. Distal convoluted tubules of sufficient length for continuous microperfusion were rarely observed in contact with the renal capsule. In three instances, in potassium-adapted animals, we were able to microperfuse this segment. These tubule segments did not secrete a significant amount of potassium. Sodium reabsorption, however, was 98 pEq/min/segment. Electron microscopic examination of the region between the puncture sites revealed that these cells were uniformly of distal cell-type morphology (Fig. 4). In addition, quantitative analysis of the distal convoluted tubule of all animals studied revealed no detectable effect of the potassium content of the diet on the structure of the distal cell type (Table 1; Fig. 5).

Studies of the connecting tubule. Connecting tubule cells dramatically increased both the basolateral membrane boundary length and surface density in response to potassium adaptation (Figs. 6, 7; Table 2). Boundary length increased by 59% from 287 ± 37 μm in control to 456 ± 26 μm in potassium-adapted animals. Basolateral membrane surface density increased by 45% from 3.25 ± 0.07 $\mu m^2/\mu m^3$ in control to 4.70 ± 0.37 $\mu m^2/\mu m^3$ in po-

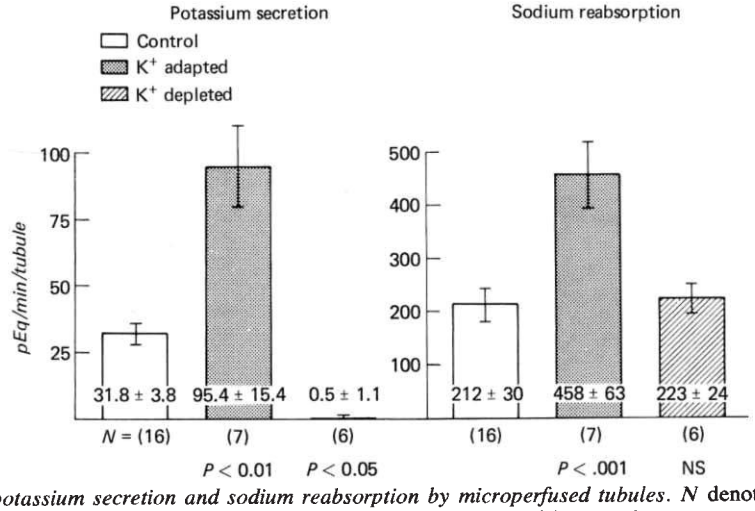

Fig. 3. *Distal nephron potassium secretion and sodium reabsorption by microperfused tubules.* *N* denotes number of distal nephrons microperfused in each group. *P* values are based on the Q test in a comparison with control.

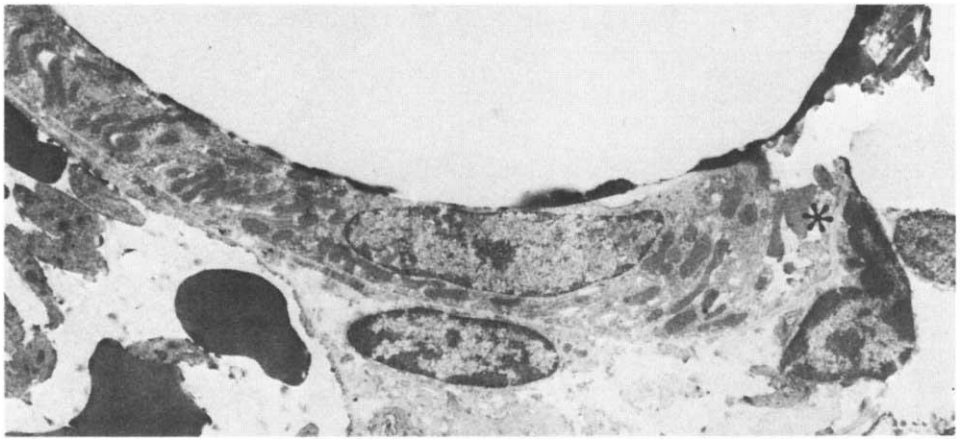

Fig. 4. *Puncture site from a microperfused distal convoluted tubule.* The collection site is indicated by a *star*. Note the flattened appearance of the distal cell due to the pressure necessary to inject microfil (located in the interstitial space and lumen). (×5500)

Table 1. Membrane morphometry of the distal convoluted tubule—Distal cell[a]

	Surface density (S_V) $\mu m^2/\mu m^3$		Cell area (A) μm^2	Boundary length (B) μm	
	Basolateral	Luminal		Basolateral	Luminal
Control ($N = 6$)	3.88 ± 0.27	0.28 ± 0.02	92.5 ± 8.7	275 ± 15	19.6 ± 1.3
K^+ adapted ($N = 6$)	4.49 ± 0.29	0.32 ± 0.06	77.3 ± 6.8	264 ± 17	18.8 ± 3.0
P	NS	NS	NS	NS	NS
K^+ depleted ($N = 6$)	4.47 ± 0.12	0.22 ± 0.01	84.8 ± 3.4	296 ± 12	14.4 ± 0.4
P	NS	NS	NS	NS	NS

[a] Values are the means ± SEM. *N* denotes the number of animals per group. P values are based on the Q test in a comparison with control.

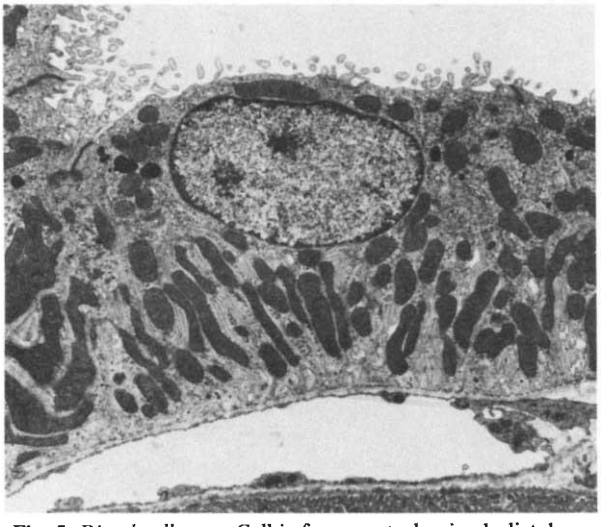

Fig. 5. *Distal cell type.* Cell is from control animal, distal convoluted tubule. This cell-type morphology was similar in potassium-depleted and potassium-adapted animals. (×4800)

tassium-adapted animals. Cell area and luminal membrane parameters were not significantly affected by potassium adaptation. In contrast, intercalated cell structure was not affected by potassium adaptation (Table 3; Figs. 6, 7). A morphometric comparison of the intercalated cell type in the connecting tubule and the initial collecting tubule indicated that the cells are structurally similar in both segments (Table 3 and Table 5). Intercalated cell incidence in the connecting tubule was 24% in control and 22% in potassium-adapted animals. These values were not significantly different from each other. Statistical analysis also indicated that intercalated cell incidence is similar in the connecting tubule and the initial collecting tubule.

Studies of the initial collecting tubule. Principal cells in the initial collecting tubule dramatically increased both the basolateral membrane boundary length and surface density in response to potassium adaptation. This striking increase in boundary length of 180%, from 162 ± 14 μm in control animals to 455 ± 45 μm in potassium-adapted animals, is presented in Figs. 8, 9, and Table 4. The increase in basolateral membrane surface density from 3.28 ± 0.08 $\mu m^2/\mu m^3$ in control to 4.77 ± 0.29 $\mu m^2/\mu m^3$ with potassium adaptation (Table 4) occurred despite an increase in cell area from 64.0 ± 5.7 μm^2 in

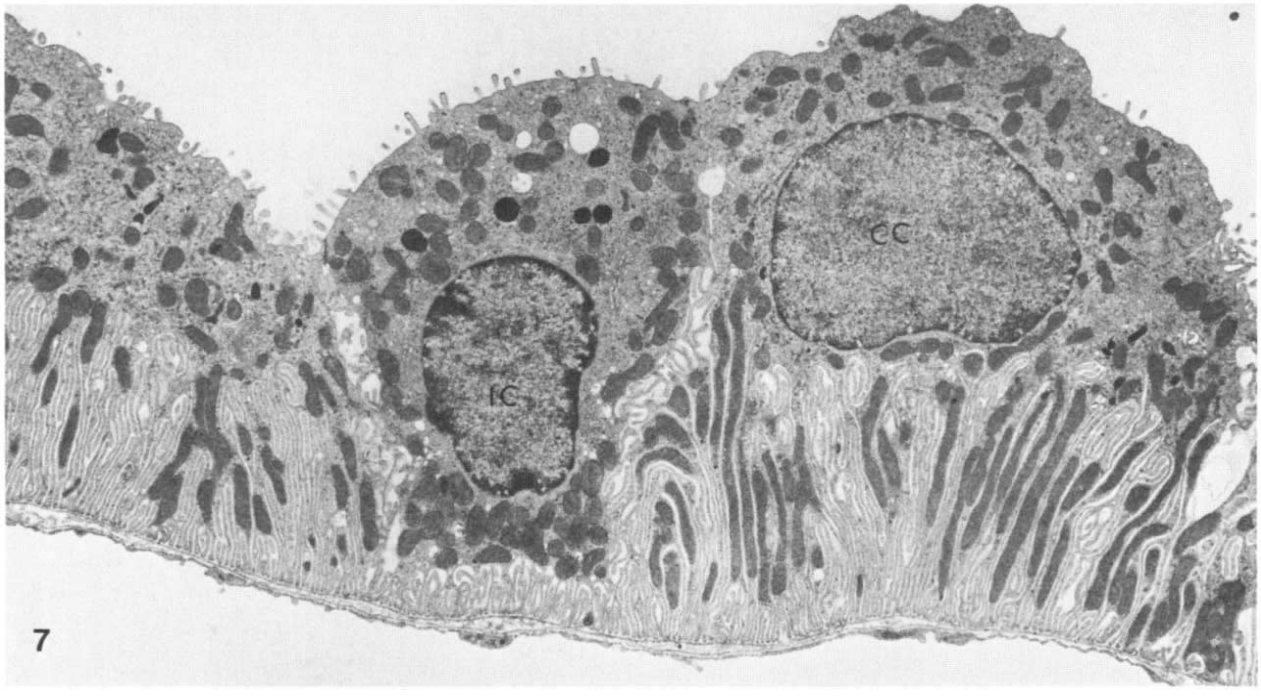

Fig. 6. *Control connecting tubule with a connecting tubule cell (CC) and an intercalated cell (IC).* Note the relatively smooth luminal membrane and the mitochondria within the basolateral infoldings of the connecting tubule cell. (×5400). **Fig. 7.** *Potassium-adapted connecting tubule.* Note the increase in the basolateral membrane area of the connecting tubule cell (CC) compared with control (Fig. 6). Intercalated cell (IC) structure is not affected by potassium adaptation. (×5700)

Table 2. Membrane morphometry of the connecting tubule—Connecting tubule cell[a]

	Surface density (S_V) $\mu m^2/\mu m^3$		Cell area (A) μm^2	Boundary length (B) μm	
	Basolateral	Luminal		Basolateral	Luminal
Control ($N = 5$)	3.25 ± 0.07	0.21 ± 0.02	102 ± 9	287 ± 37	16.8 ± 0.4
K^+ adapted ($N = 5$)	4.70 ± 0.37	0.18 ± 0.01	125 ± 7	456 ± 26	18.2 ± 1.1
P	<0.01	NS	NS	<0.01	NS

[a] Values are the means ± SEM. N denotes the number of animals per group. P values are based on the Q test in a comparison with control.

Fig. 8. *Control initial collecting tubule.* Note the variability in intercalated cell (IC) morphology. A principal cell (PC) is between the intercalated cells. (×5600). **Fig. 9.** *Potassium-adapted initial collecting tubule.* Both principal cells (PC) have extensive infoldings of the basolateral membrane compared with control principal cells (see Fig. 8). Occasional mitochondria are located within the basolateral infolds (compare with connecting tubule cells in Figs. 6 and 7). Intercalated cell (IC) morphology is similar to control (×5200). The *star* marks the renal capsule.

Table 3. Membrane morphometry of the connecting tubule—Intercalated cell[a]

	Surface density (S_V) $\mu m^2/\mu m^3$		Cell area (A) μm^2	Boundary length (B) μm	
	Basolateral	Luminal		Basolateral	Luminal
Control ($N = 5$)	1.60 ± 0.25	0.54 ± 0.14	60.5 ± 10.4	69.8 ± 2.2	24.6 ± 5.6
K^+ adapted ($N = 5$)	1.42 ± 0.09	0.53 ± 0.11	47.2 ± 0.4	52.7 ± 3.4	19.7 ± 3.8
P	NS	NS	NS	NS	NS

[a] Values are the means ± SEM. N denotes the number of animals per group. P values are based on the Q test in a comparison with control.

Table 4. Membrane morphometry of the initial collecting tubule—Principal cell[a]

	Surface density (S_V) $\mu m^2/\mu m^3$		Cell area (A) μm^2	Boundary length (B) μm	
	Basolateral	Luminal		Basolateral	Luminal
Control ($N = 6$)	3.28 ± 0.08	0.33 ± 0.2	64.0 ± 5.7	162 ± 14	16.0 ± 0.9
K^+ adapted ($N = 6$)	4.77 ± 0.29	0.26 ± 0.02	115 ± 10.0	455 ± 45	22.5 ± 1.8
P	<0.01	NS	<0.01	<0.01	<0.01
K^+ depleted ($N = 6$)	2.60 ± 0.17	0.27 ± 0.01	72.4 ± 8.5	155 ± 26	15.0 ± 1.0
P	NS	NS	NS	NS	NS

[a] Values are the means ± SEM. N denotes the number of animals per group. P values are based on the Q test in a comparison with control.

control to 114.6 ± 4.9 μm^2 in potassium adaptation.[2] We also observed a small but statistically significant increase in the luminal membrane boundary length of the principal cell type from 16.0 ± 0.9 μm in control to 22.5 ± 1.8 μm in potassium-adapted animals (Table 4). In contrast to the very profound structural changes associated with potassium adaptation, we observed no effect of the potassium-depleted diet on the principal cell type morphology (Table 4, Fig. 10).

We noted some variation in the morphology of the intercalated cell type, which can be seen in Fig. 8. Some intercalated cells contain numerous mitochondria and abundant luminal membrane microplicae. Other intercalated cells are characterized by numerous apical cytoplasmic vesicles. This observation confirms previous reports by Kriz et al [3, 5] and Crayen and Thoenes [4] that describe two forms of intercalated cells in the distal nephron. In our morphometric analysis, we did not subdivide intercalated cells into groups because as Kaissling and Kriz [5] point out, there are many transitional cells that would make separation arbitrary.

Intercalated cells of the initial collecting tubule were not affected by the diets (Table 5, Figs. 8, 9, 10). Surface density, boundary length, and cell area in potassium-adapted and potassium-depleted animals were similar to control values. The incidence of intercalated cells was 32% of total cells in control, 32% in potassium-adapted, and 38% in potassium-depleted animals. These values were not significantly different from each other. The total number of cells, seen in cross section, was about 10 for all three groups of animals.

These morphologic changes, limited to the principal cell and connecting tubule cell type, correlated with a 200% increase in distal nephron potassium secretion (Fig. 3). Because the early distal convoluted tubule did not secrete potassium, we conclude by exclusion that cells of the connecting tubule and the initial collecting tubule are responsible for distal nephron potassium secretion.

Discussion

Numerous micropuncture and microperfusion studies have demonstrated that the distal nephron and the collecting tubules regulate the amount of potassium appearing in the final urine (reviewed in Refs. 26-28). Although some investigators have recently questioned whether there is net transport of potassium by the superficial distal nephron under control conditions [21], there is general agreement that potassium secretion by this segment is enhanced by a variety of conditions, including potassium adaptation [17]. Secretion of potassium is abolished by potassium depletion [30, 31].

The mechanism of transepithelial potassium transport by the distal nephron has been extensively examined. Basolateral membrane potassium uptake has been shown to be an important determinant of transepithelial potassium transport [32]. Several lines of evidence indicate that Na-K-ATPase, located in the basolateral membrane, is involved in potassium secretion by the distal nephron. In a recent publication, Katz, Doucet, and Morel [33] have found a high activity of Na-K-ATPase in the distal convoluted tubule of the rat nephron and much lower, but significant, activities in cortical and medullary collecting tubules. Stimulation of renal potassium excretion by potassium adaptation is associated with increased activity of Na-

[2] Because measurements of transport in renal tubules are a function of tubule length, it is clearly desirable to express morphologic measurements as surface area per millimeter of tubular length. Unfortunately, because of the unoriented nature of tubules in the cortex of the intact kidney, there is no rigorous way of obtaining such values. From perpendicular cross-sections of six initial collecting tubules from control animals, we estimate that the luminal membrane surface area is on the order of 2×10^5 μm^2/mm of tubular length and basolateral membrane about 14×10^5 μm^2/mm of tubular length. Corresponding estimates for six distal convoluted tubules are 1.7 and 24×10^5 μm^2/mm of tubular length for luminal and basolateral membrane, respectively.

Fig. 10. *Potassium-depleted initial collecting tubule.* Cell morphology is similar to control initial collecting tubule. PC is principal cell; IC is intercalated cell. (×5100)

Table 5. Membrane morphometry of the initial collecting tubule—Intercalated cell[a]

	Surface density (S_V) $\mu m^2/\mu m^3$		Cell area (A) μm^2	Boundary length (B) μm	
	Basolateral	Luminal		Basolateral	Luminal
Control ($N = 6$)	1.71 ± 0.16	0.50 ± 0.08	51.9 ± 4.4	70.2 ± 8.9	19.7 ± 3.1
K^+ adapted ($N = 6$)	1.48 ± 0.09	0.41 ± 0.04	55.6 ± 2.8	64.4 ± 4.8	17.5 ± 2.0
P	NS	NS	NS	NS	NS
K^+ depleted ($N = 6$)	1.94 ± 0.11	0.51 ± 0.09	52.8 ± 4.6	79.6 ± 5.4	21.4 ± 4.9
P	NS	NS	NS	NS	NS

[a] Values are the means ± SEM. *N* denotes the number of animals per group. *P* values are based on the Q test in a comparison with control.

K-ATPase in both cortical and medullary collecting tubules [34]. Modulation of potassium transport associated with changes in ATPase activity might occur by a change in basolateral membrane area. In view of the marked segmental and cellular heterogeneity in the distal nephron, it is important to determine if all cells along the tubule participate equally in potassium secretion and in modulation of transport associated with changes of dietary potassium.

The present study reports for the first time results from continuous microperfusion of the distal convoluted tubule portion of the superficial distal nephron. Because of the difficulty in finding long enough portions of this segment in contact with the renal capsule for successful microperfusion and the necessity of identifying these sites by electron microscopy, microperfusion results were only obtained from three tubules in potassium-adapted animals. Although a limited number of studies were performed, these results indicate that even in the potassium-adapted rat the distal convoluted tubule does not secrete potassium.[3] This conclusion is supported by the observation of Malnic et al [29] that the "early distal" segment (undoubtedly the distal convoluted tubule) rarely elevated luminal potassium concentration above plasma values despite a variety of maneuvers that increased late distal potassium to as much as 156% of control values. In addition, we have demonstrated that distal tubule cell structure was not affected by either diet. Pfaller et al [35] have studied distal nephron cells in potassium-adapted rats and also found no change in membrane area. They did not distinguish, however, between the distal convoluted tubule and the morphologically very different initial collecting tubule.

Several recent studies have examined the relationship between renal cell morphology and tubular

[3] Our failure to detect potassium secretion cannot be attributed to methodologic limitations. The calculated contact time (less than 1 second) was sufficient to achieve a steady-state potassium concentration in tubule fluid. Although the perfused segments were short (about 250 μm), our methods would have easily detected the amount of potassium secretion predicted for this length of tubule.

potassium transport in an attempt to elucidate the participation of each cell type in potassium transport. Wade et al [13] found an increase in basolateral membrane surface area in rabbit cortical collecting tubules associated with chronic DOCA treatment. DOCA pretreatment has been shown to increase the potassium secretory capacity of the collecting tubule [36, 37]. In potassium-adapted rats, Rastegar et al [14] found a similar increase in the rat medullary collecting duct. In both studies, the increase in basolateral membrane was limited to principal cells. In the present study, we observed, in the connecting tubule and in the initial collecting tubule, a dramatic increase in basolateral membrane boundary length and surface density in potassium-adapted animals. This change in the basolateral membrane was limited to the connecting tubule cell type and the principal cell type and correlated with a 200% increase in potassium secretion by the distal nephron.

A relatively small increase (41%) in principal cell luminal membrane boundary length was also found in our studies. Although it is conceivable that changes in the luminal membrane may play a role in modulating the rate of potassium secretion, the luminal membrane surface density (S_V) was not significantly altered by potassium adaptation due to the increase in cell area. Rastgar et al [14] have recently found similar changes in papillary cells. The luminal membrane boundary length and surface density of connecting tubule cells, however, did not increase in response to potassium adaptation (Table 2). Therefore, the possible physiologic significance of this luminal membrane change, limited to principal cells, is uncertain. Nevertheless, the fact that morphologic changes were specific for connecting tubule cells and for principal cells and were not identified in either distal cells or intercalated cells suggests that the connecting tubule cell and the principal cell mediates potassium secretion in the distal nephron.

It is presently impossible in the rat to examine directly the participation of the connecting tubule in potassium transport. Connecting tubules are short, usually below the renal capsule, and therefore inaccessible to micropuncture. This segment, however, has been studied in the rabbit by Shareghi and Stoner [8], and they found that both the connecting tubule and the cortical collecting tubule secrete potassium. Kriz and Kaissling (personal communication) have found that the basolateral membrane area of connecting tubule cells is increased in the rabbit after potassium adaptation. These studies demonstrate the ability of the rabbit connecting tubule to secrete potassium and to respond structurally to potassium adaptation.

In recent years, there has been a reexamination of the issue of whether physiologic conditions alter the number of intercalated cells in collecting tubules. In an early light microscope study of the renal medulla, Oliver et al [38] proposed that the intercalated cell population was labile and that the incidence of these cells increased after potassium depletion. Other experimental treatments have also been reported to increase the number of intercalated cells [39]. An extensive recent study by Hansen et al [40] found, however, no change in the incidence of intercalated cells after a variety of maneuvers that both stimulated and inhibited potassium and hydrogen transport. Our electron microscope observation that intercalated cells represent about 32 to 38% of the cells in the initial collecting tubule, regardless of the potassium content of the diet, is consistent with the results of Hansen, Tisher, and Robinson [40]. The study of the medullary collecting duct in potassium-depleted rats by Stetson et al reached the same conclusion [15]. But, by using freeze-fracture electron microscopy, Stetson et al were able to distinguish two different forms of the intercalated cell type and noted a shift from one form to the other with potassium depletion. In addition, Rastegar et al [14] have reported a decrease in the number of intercalated cells with microplicae in potassium-adapted animals, but they did not suggest a change in the total number of intercalated cells. It is possible that by failing to recognize both forms of the intercalated cell, early workers interpreted shifts in morphology as a change in number of cells, leading to the suggestion that principal cells could become intercalated cells. Current evidence (present study and Refs. 5, 14, 15, 40) favors the view that at least in the rat the intercalated cells are a discrete and relatively constant cell population from the connecting tubule through the outer medullary collecting tubule, but that changes in their structure can occur. Because workers have consistently noted changes in the structure of intercalated cells in the outer medulla with potassium depletion [15, 38, 41], it has been suggested that this cell type may play a role in potassium reabsorption in this segment.

We found no morphologic change in intercalated cells of the initial collecting tubule either after potassium depletion or potassium adaptation. Although the lack of an effect of potassium depletion is in contrast to observations in the medullary col-

lecting tubule, Toback et al [41] noted that only the cells in the medulla responded to potassium depletion, and that intercalated cells in the cortex were normal. Therefore, it appears that intercalated cells in the medullary collecting tubule and initial collecting tubule do not respond structurally in a similar fashion to potassium depletion. Potassium transport also appears to be different in the two regions during potassium depletion. Even though our microperfusion measurements and previous micropuncture studies [30, 31] show no net reabsorption of potassium by the superficial distal nephron, indirect evidence indicates that the medullary collecting tubule may reabsorb a substantial amount of potassium during potassium depletion [30, 31, 42]. Therefore, there is good evidence that the intercalated cells of the initial collecting tubule and medullary collecting tubule respond both structurally and physiologically in a different fashion to potassium depletion.

Conclusion. Potassium secretion by the superficial distal nephron occurs not in the distal convoluted tubule but in the connecting tubule and the initial collecting tubule.[4] The increase in basolateral membrane area in the connecting tubule cell and the principal cell suggests that these cell types are responsible for potassium secretion. Changes in membrane area and the associated increase in Na-K-ATPase appears to play a role in the mechanism by which distal nephron cells increase potassium transport in potassium adaptation.

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[4]Recently, we successfully microperfused the last half of the superficial nephron. The concentration of potassium secreted by this region alone was equal to that secreted by the entire accessible distal nephron, thereby supporting our conclusion.

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